THE PHILIPPINE
JOURNAL OF SCIENCE
B. TROPICAL MEDICINE
VOL. XI JULY, 1916 No. 4

THE RELATION BETWEEN THE AMOUNT OF CHOLERA CULTURE INJECTED INTO THE GALL BLADDER AND THE STATE OF CHOLERA CARRIERS IN EXPERIMENTAL ANIMALS

By OTTO SCHÖBL
(*From the Biological Laboratory, Bureau of Science, Manila, P. I.*)

In the work on experimental cholera carriers the following question naturally presented itself: What relation exists between the amount of cholera vibrios injected into the gall bladder and the state of cholera carriers in experimental animals? In previous experiments [1] the amount injected was considerable—that is, one tenth of one slant. Under such conditions all inoculated guinea pigs were positive up to the thirteenth day, inclusive. It remained to be decided whether the number of "takes" would be as high as that, if a smaller amount of cholera culture be injected, and whether or not the duration of the carrier state would be affected by the variation of the amount of cholera injected into the gall bladder. The following experiments were arranged so that amounts of cholera culture varying from 1/80 to 1/100,000,000 of a slant were injected. The animals of one lot were killed [2] on the seventh and those of another lot on the thirteenth day after inoculation.

From these experiments it is evident that the amount of cholera culture injected into the gall bladder can be decreased considerably and still all of the inoculated animals will become carriers. In our experiments the inoculum was decreased from 1/80 to 1/80,000 of one slant. The percentage was just as high as it was when 1/10 of a slant was inoculated. When the inoculum was diluted beyond this limit, the results became irregular. Animals that received 1/100,000 and 1/10,000,000 of a slant, respectively, were found negative, while the animal that was inoculated with 1/100,000,000 of a slant was found positive.

[1] *Journ. Inf. Dis.* (1916), 18, 307–314. [2] Op. cit.

TABLE I.—*Showing the number of takes in experimental cholera carriers after intravesicular inoculation of decreasing doses of cholera culture.*

[Animals killed seven days after inoculation.]

Dilution.	Direct plates.				Peptone cultures.			
	Gall bladder.	Duodenum.	Ileum.	Cæcum.	Gall bladder.	Duodenum.	Ileum.	Cæcum.
1/80	n	f	n	vn	+	+	+	+
1/160	vn	f	f	—	+	+	+	—
1/320	vn	vf	vf	—	+	+	+	—
1/640	vn	f	vf	—	+	+	+	+
1/1,000	n	vf	n	f	+	+	+	+
1/2,000	n	vf	f	—	+	+	+	—
1/4,000	vf	—	—	—	+	+	+	—
1/8,000	vf	—	—	—	+	+	+	+
1/20,000	n	vf	vf	—	+	+	+	—
1/40,000	vf	—	—	—	+	+	+	—
1/80,000	n	f	f	—	+	+	+	—
1/100,000	—	—	—	—	—	—	—	—
1/10,000,000	—	—	—	—	—	—	—	—
1/100,000,000	vn	n	vn	f	+	+	+	+

+=cholera vibrios found; —=cholera vibrios not found; vf=less than half a dozen colonies; f=about one dozen colonies; n=about 200 colonies; vn=more than 200 colonies.

TABLE II.—*Showing the duration of carrier state in experimental animals after intravesicular injection of decreasing doses of cholera culture.*

[Animals killed thirteen days after inoculation.]

Dilution.	Direct plates.				Peptone cultures.			
	Gall bladder.	Duodenum.	Ileum.	Cæcum.	Gall bladder.	Duodenum.	Ileum.	Cæcum.
1/80	f	—	—	—	+	+	+	—
1/160	f	—	—	—	+	+	+	—
1/320	vf	—	—	—	+	—	+	—
1/640	f	—	—	—	+	+	+	—
1/1,000	f	vf	f	—	+	+	+	—
1/2,000	n	vf	(a)	—	+	+	+	—
1/4,000	f	—	—	—	+	+	+	—
1/8,000	n	f	f	—	+	+	+	+
1/20,000	vf	—	—	—	+	+	+	—
1/40,000	—	—	—	—	—	—	—	—
1/100,000	—	—	—	—	—	—	—	—
1/100,000,000	—	—	—	—	+	+	+	+

[a] Vibrios present, but overgrown by other bacteria.

Table II shows the duration of carrier state in animals inoculated with decreasing amounts of cholera culture. All of the carriers that were infected with from 1/80 to 1/20,000 of a slant lasted the usual length of time, which was found in our

previous experiments[3] to be thirteen days. Animals injected with 1/40,000 and 1/100,000 of a slant were found negative. Again the animal that received the smallest dose, 1/100,000,000, still harbored cholera vibrios on the thirteenth day.

CONCLUSIONS

1. Inoculation of a relatively small amount of cholera vibrios into the gall bladder may produce carriers in animals, but the results are not as regular as they are when larger amounts are used for inoculation.

2. The amount of inoculum seems not to have any direct bearing on the duration of the carrier state in animals according to the preceding experiments. The negative animals which were inoculated with 1/40,000 and 1/100,000 are to be interpreted as failure "takes," as the animal that received a far smaller dose was positive.

[3] Op. cit.

THE INFLUENCE OF BILE UPON THE DURATION OF THE STATE OF CHOLERA CARRIERS IN EXPERIMENTAL ANIMALS

By OTTO SCHÖBL

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

In order to gain some information with regard to the relation of cholagogs to the state of cholera carriers, some experiments were arranged in the following way.

Thirteen guinea pigs of the same size were inoculated simultaneously. One tenth of one slant of cholera culture was injected into the gall bladder.[1] After the inoculation seven of the animals were given 1 cubic centimeter of ox bile by mouth every third day. They were killed and examined at intervals as indicated in Table I. The remainder of the experimental carriers, six in number, which received no bile, were taken as controls.

TABLE I.—*Showing the influence of bile upon the duration of the state of cholera carriers in experimental animals.*

TREATED ANIMALS.

Killed after inoculation.	Direct plates.				Peptone cultures.			
	Bile.	Duodenum.	Ileum.	Cæcum.	Gall bladder.	Duodenum.	Ileum.	Cæcum.
Days.								
16	n	—	f	—	+	—	+	—
16	n	—	f	—	+	—	+	+
19	n	f	f	—	+	+	+	+
19	vn	vf	n	—	+	+	+	+
21	n	—	n	—	+	—	+	—
28	n	—	n	—	+	—	+	—
35	n	f	f	—	+	+	+	+

UNTREATED CONTROLS.

Days.								
16	n	—	vf	—	+	+	+	+
16	—	—	—	—	—	—	—	—
19	n	—	f	—	+	+	+	+
21	—	—	—	—	—	—	—	—
28	f	—	—	—	+	—	—	—
35	—	—	—	—	—	—	—	—

+ = cholera vibrios found; — = cholera vibrios not found; vf = less than half a dozen colonies; f = about one dozen colonies; n = about 200 colonies; vn = more than 200 colonies.

[1] For technique, see *Journ. Inf. Dis.* (1916), 18, 307–314.

Seven of the seven experimental cholera carriers fed on bile were found positive from sixteen to thirty-five days after the inoculation, while only three of the six control carriers were found to harbor cholera vibrios during the same period of time.

These experiments show clearly that the increased flow of bile does not further the disappearance of the cholera vibrios from the gall passages and from the intestine. On the contrary, it seems strongly to indicate that the administration of bile, a cholagog "par excellence," tends to prolong rather than to shorten the duration of the state of cholera carrier.

CARBOHYDRATE FERMENTATION BY BACILLUS PESTIS, COMPARING CERTAIN AMERICAN AND ORIENTAL STRAINS

WITH ANALYSIS OF DISCREPANCIES OF FERMENTATIONS WITH HISS'S SERUM WATER, LITMUS AGAR, AND BOUILLON [1]

By H. WINDSOR WADE

(*From the Biological Laboratory, Bureau of Science, Manila, P. I.*)

In spite of the great amount of study to which *Bacillus pestis* has been subjected, it cannot be determined positively by a survey of the literature what ability this organism has for breaking down carbohydrates with the production of acid, nor is it clear whether all strains are alike in this regard.

Recently the opportunity has arisen to compare cultures isolated in New Orleans, Louisiana, in 1914, and which at the time were thought possibly to be different from those from the Orient, with a number of strains isolated at different times in the Philippine Islands. As a result of a somewhat intensive study, which was extended considerably beyond the scope at first intended, the fermentative powers of these strains have been clearly defined. The results are recorded, not because of any controversy on the subject, but to establish clearly these features of the biology of *Bacillus pestis* and to emphasize the unusual similarity of the strains studied, as well as to illustrate certain features of fermentation reactions in general.

LITERATURE

Few of the many articles touching on the bacteriology of plague deal with the reactions of the organism in carbohydrate media. The earlier writers knew that acid was produced in dextrose media, and in 1898 Gioso and Biginelli (1) determined this to be lactic acid. Rees (2) said that, according to Klein, *B. pestis* formed a small amount of acid in sugar agar and caused coagulation of milk in about two weeks. Other authors, however, stated that litmus milk was little affected. The German Plague Commission (1897–1898), according to Dieudonné and Otto, (3) tested for gas formation in bouillons containing dextrose, levulose, lactose, and mannite, with negative results. Simple acid formation

[1] Received for publication August 25, 1916.

was not considered an important feature of bacterial biology at that period. The Indian Plague Commission found, according to the same authors, that acid was formed in dextrose, levulose, mannite, and galactose, but not in lactose and dulcite. Calvert(4) spoke of reddening of litmus glucose agar. Herzog(5) made the statement that it does not ferment dextrose, levulose, lactose, or mannite. He did not say whether this was the result of his own observations nor whether the term "ferment" was intended to signify gas production. It is improbable that he tested for acidification in these media.

The first extended tests were those of MacConkey(6) in 1905. In his bile salt medium for a base he obtained acid without gas in dextrose, levulose, maltose, galactose, mannite, and dextrin, but no change in raffinose, lactose, saccharose, dulcite, and sorbite. It is not stated how this acidity was determined. Wherry(7) described the organism isolated from a plague rat as growing only in the open end of fermentation tubes containing 1 per cent dextrose, levulose, lactose, saccharose, and starch bouillons. These cultivations were intended to rule out *B. coli,* and no mention of acid formation was made. Later, however,(8) he described strains from human cases and from ground squirrels, all of which produced acid but no gas in 1 per cent dextrose, levulose, galactose, maltose, and mannite broths, but no change in lactose, saccharose, or inulin. Vourland(9) tested a single strain and found that beside the sugars given by MacConkey glycerin, arabinose, xylose, salicin, saccharose, and lactose gave acid reactions. Dextrin, however, was not acidified, and he noted that saccharose reverted to blue.

MacConkey,(10) in 1908, reiterated his previous results, adding adonite, inulin, amygdalin, and α-methyl glucoside to his list of unaffected substances, and suggested that Vourland's results might have been due to a possible trace of muscle sugar in the ordinary nutrient agar which he employed. The Advisory Committee(11) confirmed some of MacConkey's reactions, having used his medium with glucose, levulose, mannite, and galactose, which gave acid, and lactose and dulcite, which were not changed.

McCoy,(11) working in the Hygienic Laboratory in Washington, determined the virulence of old strains from Manila, Bombay, Jedda, New York, Glasgow, San Francisco, and Reedy Island in comparison with newly isolated strains from San Francisco. The old strains had been tested culturally by Wherry, who had found that the activity of the cultures was alike throughout—dextrose, levulose, and galactose being most actively fermented, mannite next, and maltose least. McCoy found no

difference between these and his new strains. Rowland(13) described the organism which he employed in vaccine work as forming acid in dextrose and mannite, but not touching lactose, saccharose, dulcite, adonite, inulin, and litmus milk.

At the International Plague Conference held in Mukden, (1911), the idea that the strain of organism in pneumonic plague differed from that ordinarily found in bubonic plague was discussed. Zabolotny(14) inclined to this view, but no definitely confirmatory evidence was adduced. No results of cultivation in sugar media were reported at the conference. In summing up the existing evidence, Strong,(15) and later Strong and Teague,(16) declared that there was no distinction between the bubonic and the pneumonic organisms demonstrable either culturally or by study of virulence and inmunology.

Schöbl,[2] working in the Bureau of Science laboratories in 1914, made parallel reactions on litmus carbohydrate agars, using seventeen reagents and twenty-one strains of *B. pestis*, including known Chinese strains and the laboratory avirulent strain. He demonstrated no qualitative fermentation differences, but obtained results somewhat at variance with those of others in that saccharose and glycerin gave acidification, as well as dextrose, levulose, galactose, maltose, mannite, and salicin. Lactose, raffinose, dulcite, dextrin, amygdalin, inulin, inosite, adonite, sorbite, and nutrose gave no acid.

The latest report of such an investigation is that of Berlin,(17) who studied fifty-five strains, employing fourteen sugars in a concentration of 1.3 per cent in agar. He obtained uniform results, all cultures giving acid in arabinose, glucose, galactose, maltose, mannite, and levulose and no change in saccharose, lactose, raffinose, starch, dextrin, inulin, dulcite, and adonite. He concluded that the age of the cultures and their virulence played no rôle in the acid formation.

The minor differences which seem apparent upon comparing various reports would appear to be due either to technical variations by different investigators, or to the existence of subspecies recognizable only by special methods of investigation. Analysis of the reports makes the latter hypothesis seem the less probable, since no one has reported variation among the cultures under his hand, no matter how widely his results differed from those of others. There seems to be no evidence of modification of fermentative power after prolonged artificial cultivation.

[2] Personal communication.

EXPERIMENTAL CULTIVATIONS

Discrepancies among the reports were noticed when, in the summer of 1914, a case of bubonic plague was encountered at the Charity Hospital in New Orleans. The organism from this and the next two cases that appeared were studied within a few days of isolation.(18) For comparison, the strain that had been isolated two years previously from a New Orleans rat, at the time of an epidemic in Havana, was tested in parallel. In a few fermentation tests with Hiss's litmus serum-waters dextrose was fermented strongly, galactose weakly, and levulose and maltose, among others, not at all. The only value of these results was the demonstration of similarity between the old rat strain and the newly isolated human strains. The inactivities noted, particularly in view of disagreement among reports, were at the time thought possibly to indicate that the New Orleans strain differed from those encountered elsewhere. It seemed that, could this be established and foreign centers of infection by plague of the same type be located, the original source of the New Orleans invasion might be traced. This possibility made the study of the organisms of rather more than academic interest. An attempt was made to secure other strains for comparative study, but this was not successful, and the subject was not pursued.

The opportunity having now arisen to do so, I have compared, under various conditions, the four New Orleans strains and six strains carried in the biological laboratory of the Bureau of Science. For convenience the organisms have been designated by letters as follows:

Strain A. From New Orleans rat, 1912.
B. From New Orleans plague case 1, 1914 (case fatal, organisms killed rats and guinea pigs).
C. From New Orleans plague case 2, 1914 (case rapidly fatal).
D. From New Orleans plague case 3, 1914 (case recovered).
E. From Iloilo, 1912.
F. Manila case.
G. Strain "Manila VIII."
H. Strain "M I."
I. Strain "M. II."
J. "Plague avirulent," laboratory strain.

Strains *E*, *F*, and *G* were furnished by Dr. J. A. Johnston; strain *J*, by Dr. O. Schöbl; and strains *H* and *I*, by Mr. A. Guzman; all of the biological laboratory, Bureau of Science. The two last-mentioned strains are said to have been isolated by

Strong in Mukden, though this may be questioned. The Philippine organisms were undoubtedly originally introduced from China. The New Orleans strain *A* was now 4 years old, and strains *B*, *C*, and *D* were 2 years old. It may be noted, as indicating the viability of these cultures, that the transplants made in February, 1916, were from agar cultures, inoculated eighteen months previously, that had been closed with sealing wax and kept at room temperature, which in New Orleans averages rather high. Strain *E* was 4 years old, and the others ranged from 2 to 5 years in age.

LITMUS SERUM-WATERS OF HISS

In the first series of cultivation Hiss's serum-water media were used as originally, horse serum being utilized instead of beef serum. The sugars were used in but 0.5 per cent strength on account of the difficulty of obtaining some of them. The total number of reagents used was nineteen. Inulin was not procurable. The results of the positive and irregular reactions in two series appear combined in Table I. For brevity the reagents not showing change by this method are listed in the note to Table I.

The reaction in dextrose was always rapid and complete, and in the mannite cultures coagulation also developed regularly, though considerably more slowly. The levulose cultures reacted with much irregularity. In arabinose there was at times fairly definite acidification, but this was slight and very inconstant in these low-sugar series. Litmus milk usually showed but a faint change. The fourteen media listed as negative, certain of which were tested repeatedly, showed absolutely no change after ten days.

A fact of interest in this series is that there was demonstrated absolutely no distinction between the New Orleans strains and those isolated in the Philippine Islands. This similarity has been found to persist with various media.

These results were so at variance with most authorities, though for the most part comparable with those obtained in New Orleans, that the study was extended to determine the reason for the discrepancies. In order to determine, for one thing, the part that low-sugar concentration played, a small series of 1 per cent media was later inoculated. The reactions obtained with these appear in Table VIII and demonstrate at least a moderate improvement over the 0.5 per cent media.

TABLE I.—*Reaction of B. pestis in Hiss's litmus serum-waters containing 0.5 per cent carbohydrate.*

[In Tables I and VIII the symbols are used as follows (compare with Table X): − = no change; ± = faint acidity, not sufficient to be consider a positive reaction; + = medium reddened, but still clear and fluid; 2 + = medium red and fluid, but with slight cloudiness; 3 + = medium opaque, but fluid; 4 + = medium coagulated. The figures in parentheses indicate the day of cultivation on which the recorded change was observed.]

Strain of organism.	Dextose.	Mannite.	Levulose. Series II.	Arabinose. Series II.[a]		Litmus milk.
				A.	B.	
A	4+ (2)	+ (1) 3+ (3) 4+ (4)	+ (1) 3+ (3) 4+ (4)	2+ (3)	± (3) 2+ (4)	±.
B	4+ (2)	+ (3) 3+ (4) 4+ (6)	Negative	+ (8)	Negative	Negative.
C	4+ (2)	± (2) 3+ (3) 4+ (4)	do	+ (3) 2+ (4)	do	Do.
D	4+ (2)	± (1) + (2) 3+ (3) 4+ (5)	+ (2) 3+ (3) 4+ (4)	+ (3) 2+ (4)	do	±.
E	4+ (2)	± (2) + (3) 3+ (4) 4+ (7)	+ (2) 3+ (5)	± (4)	do	±.
F	4+ (2)	+(1) 3+ (4) 4+ (7)	+ (3) 3+ (4)	+ (4)	do	±.
G	4+ (2)	+(1) 2+ (3) 3+ (4) 4+ (5)	+ (4) 3+ (6)	+ (3)	do	±.
H	4+ (2)	+(1) 2+ (3) 3+ (4) 4+ (5)	2+ (3) 4+ (4)	+ (3) 2+ (4)	± (6)	±.
I	4+ (2)	+(1) 3+ (3) 4+ (5)	+ (2) 2+ (4)	Negative	Negative	±.

NOTE.—The following sugars were not fermented in these series: Maltose, galactose, salicin, glycerin, saccharose, lactose, dextrin, nutrose, dulcite, adonite, raffinose, sorbite, inosite, amygdalin, and arabinose.

[a] Results of successive sets with the same lot of medium.

LITMUS-SUGAR AGARS

To control the results obtained with serum waters, a parallel set of cultures was made using litmus-sugar agar slants. In Table II are shown details of reactions in the five media which, of the eighteen used in the primary 0.5 per cent set, were acidified. Later, on account of the results with bouillon titration, a 1 per

cent set was cultivated, using only the weakly or irregularly fermented substances—salicin, arabinose, galactose, maltose, and glycerin. The reactions with these are included in the table. The stages of acidification are approximately indicated in a manner which is of use for purposes of comparison.

TABLE II.—*B. pestis on litmus-sugar agars (incubation at 37° C. for ten days).*

[In Table II the symbols used are as follows: + = slight reddening of agar beneath the growth; 2 + = a spreading area of acidification; 3 + = diffusion of acid not quite complete; 4 + = complete reddening of the entire slant. The figures in parentheses indicate the days after inoculation on which the changes were noted.]

	Series I. 0.5 per cent.					Series II. 1 per cent.				
	Dextrose.	Mannite.	Levulose.	Maltose.	Glycerin.	Maltose.	Glycerin.	Arabinose.	Galactose.	Salicin.
A	4+(2)	4+(2)	4+(4)	4+(2)	—	4+(7)	—	4+(3)	4+(5)	4+(7)
B	4+(2)	4+(2)	4+(2)	(a)	—	4+(10)	—	4+(3)	4+(5)	4+(5)
C	4+(2)	4+(2)	4+(3)	(a)	—	3+(10)	—	4+(3)	4+(5)	4+(5)
D	4+(2)	4+(2)	4+(2)	(a)	—	4+(7)	—	4+(3)	4+(5)	4+(7)
E	4+(3)	4+(2)	4+(4)	4+(3)	—	4+(5)	—	4+(5)	2+(10)	4+(5)
F	4+(3)	4+(3)	4+(3)	±(1) +(2) 2+(3) +(6) —(7)	—	4+(4)	—	4+(5)	2+(10)	4+(5)
G	4+(4)	4+(3)	4+(4)	4+(4)	4+(5)	4+(5)	4+(10)	4+(5)	1+(2) 2+(3) 1+(4) —(5) +(7) 2+(10)	(a)
H	4+(3)	4+(3)	4+(3)	±(1) 2+(2) —(8)	4+(6)	4+(6)	4+(10)	4+(3)	4+(7)	(a)
I	4+(2)	4+(2)	4+(3)	4+(4)	—	4+(4)	—	4+(4)	2+(10)	4+(7)

NOTE.—Reagents unfermented in the 0.5 per cent series: Arabinose, galactose, salicin, saccharose, amygdalin, dextrin, sorbite, inosite, lactose, raffinose, adonite, dulcite, and nutrose. Arabinose and galactose were fermented in the 1 per cent series.

[a] Negative in two tests with this lot of medium.

In the 0.5 per cent agars dextrose and mannite were as promptly and as completely acidified as in the serum waters. Levulose, however, showed a constant and complete acidification, and maltose was much more markedly changed, though with the three New Orleans human strains there was no reaction. With two of the strains obtained locally (*F* and *H*), there was a partial acidification with subsequent reversion; in both instances the reddening occurred only after a heavy, primarily nonacid-producing growth had developed, and then was confined to the

medium under small parts of the growth. That this localized acid production was temporary is indicated by the fact that when sufficient time had elapsed for the reaction of the medium to become uniform throughout by diffusion the entire slant again became blue.

With 1 per cent agars the strains inactive in series I showed fermentation. Arabinose cultures also showed more marked acidity, but the galactose reactions were still irregular and not satisfactory. With salicin there was complete inactivity with three strains, while the glycerin cultures, instead of reacting more rapidly, were somewhat delayed in attaining complete change. These results emphasize the fact that litmus-sugar agars, even of 1 per cent concentration, are not satisfactory for the demonstration of acid production by organisms such as *B. pestis*, the fermentation activities of which are of low degree.

SUGAR BOUILLONS, PHENOLPHTHALEIN TITRATIONS

In order to establish quantitatively the fermentative power of the different strains, and also to determine the relation between actual acid production by *B. pestis* and the indication of such activity given by the media previously employed, a complete series of broth cultures was made in bouillons containing the eighteen carbohydrates previously used.

Each tube contained from 15 to 20 cubic centimeters of 0.5 per cent sugar bouillon, made with beef extract (Liebig's) in the ordinary manner. This was used because the pest organism grows fairly well in it and because preliminary tests had shown that no change in reaction detectable by titration occurs from growth of *B. pestis* in broth so made. The tubes of the first series contained small, inverted "fermentation tubes." After three days, no gas having appeared, these were removed. After ten days of total incubation they were heated in an Arnold sterilizer for twenty minutes before being titrated. The estimations were made, using N/20 and N/50 sodium hydroxide, respectively, in the two series tested. Hot titration was used by choice, in spite of the fact that it gives somewhat higher readings than are obtained in the cold. Accuracy of comparison was considered of first importance rather than the exact determination of absolute acid increase.

Two series were titrated, as upon titration the first lot of media was found to have been between 1.5 and 2.2 per cent acid. The results obtained in series I agree in the main with those of the second series, which was adjusted to about 0.3 per cent acid before sterilization.

In Table III are shown the amounts of acid increase by each strain in those sugar bouillons of series II that were in any degree affected. There is also shown the average per cent of acid production in each sugar, with the similar averages of series I appended for comparison. Further, as indicating the relative fermentative activities of the different strains, the average acid production by each strain of organism in all but the glycerin medium is shown.

TABLE III.—*Increase of acidity in 0.5 per cent sugar bouillons (series II) in cubic centimeters of normal sodium hydroxide.*

Strain.	Dextrose.	Mannite.	Levulose.	Maltose.	Salicin.	Arabinose.	Galactose.
A	1.5	1.7	1.3	1.2	1.8	1.2	1.2
B	1.5	1.7	1.4	0.8	1.8	1.0	0.9
C	1.6	1.5	1.2	1.1	1.8	1.0	0.8
D	1.5	1.6	1.6	0.6	1.9	1.0	0.8
E	1.4	1.7	0.8	1.1	1.6	1.1	0.8
F	1.4	1.4	1.3	1.1	1.6	0.8	0.9
G	1.5	1.3	1.1	1.0	0.4	1.2	0.3
H	1.4	1.5	1.1	1.0	1.1	1.2	0.7
I	1.7	1.7	(a)	1.1	1.8	1.3	0.9
Average increase	1.50	1.57	1.22	1.00	1.53	1.09	0.80
Average increase, series I	1.22	1.02	1.64	0.82	0.81	0.79	0.52

Strain.	Dextrin.	Inosite.	Sorbite.	Saccharose.	Amygdalin.	Glycerin (1 per cent).	Average activity.
A	0.5	0.1	0.1	0	0	0.6	0.82
B	0.4	0.1	0.2	0	0	0.1	0.75
C	0.5	0	0.1	0	0	0	0.74
D	0.5	0	0.1	0.1	0	0.2	0.75
E	0.5	0	0.2	0	0	0.1	0.71
F	0.5	0	0.1	0.2	0.1	0.1	0.72
G	0.4	0	0	(b)	0	1.1	0.60
H	0.4	0	0.1	0	0	0.4	0.67
I	0.5	0	0.3	0.3	0.3	0.2	0.84
Average increase	0.47	0	0	0	0	(c)	0.73
Average increase, series I	0.35	0.25	0.31	0.41	0.37	.....	.....

[a] No growth. [b] Contaminated. [c] Irregular.

As a result of these titrations the reagents used may be roughly grouped according to the extent to which *B. pestis* produced acid in bouillons containing them. Group I includes dextrose, mannite, and levulose, which are strongly acidified. In group II are salicin, arabinose, maltose, and galactose, which are weakly acidified. Glycerin holds an odd position in that but two of the

strains here used (*G* and *H*) ferment it. To these should be added strain *J*, which was obtained after this phase of the work was completed, but which shows acid production in other glycerin media. The reaction with dextrin may be due to traces of dextrose; at any rate, being less than 0.5 per cent, it is so slight as to seem negligible.

With regard to the second group it is interesting to note that in spite of the constantly negative results in the litmus media with all of these but maltose, and the negative serum water and irregular agar reactions even with this sugar, their bouillons show a constant, regular acidification ranging from 0.6 to 1.9 per cent.

While there is no distinction between American and Philippine strains, there will be noted in the averages slight variations in the total acid production by the different strains. Thus the old New Orleans rat strain (*A*) and one of the Bureau of Science strains (*I*) cause considerably higher acidification than the others, while one of the Philippine strains in particular (strain *G*) shows a constantly comparatively low acid tolerance.

INFLUENCE OF SUGAR AND MEDIUM ON MORPHOLOGY

The morphology of *B. pestis* has been so often and so exhaustively described that but a few points in connection with the influence of the different sugars used merit brief notice.

Three-day sugar-agar cultures.—All smears examined were stained with Loeffler's blue. On dextrose, mannite, and levulose, in which media fermentation begins promptly, smears uniformly show much degeneration and involution. When acid production appears late, the degeneration and involution occur to a less degree. The cultures on sugars not acidified give well-stained organisms, though variation in morphology in different instances is marked. The same sugar, however, seems in the majority of instances to produce somewhat similar effects in the different strains. Salicin, for instance, usually produces long, often thready and at times almost filamentous organisms (*B. proteus* type), not at all recognizable as *B. pestis*. Dextrin and arabinose also show this tendency. On the other hand, glycerin induces fairly constantly the formation of short, chunky, deeply staining bacilli, often showing the typical bipolar appearance of *B. pestis* in exudates. In a very few instances there were encountered long pale organisms with deeply stained polar bodies, indistinguishable from *B. diphtheriæ* under the blue stain.

Ten-day sugar-bouillon cultures.—The morphology in dextrose and mannite bouillons differs as widely from that on the similar

agars as do the quantities of growth on these media. Here is found with regularity extensive, typical chain formation, often with an almost sheathlike capsular layer. In media containing substances such as glycerin and arabinose, which are not fermented and in which growth is not heavy, chain formation is not a feature and loose bacillary forms predominate. Stalactites do not develop in such media. Galactose bouillon, it may be noted, produces typical, short, bipolar forms rather more constantly than any other. Filamentous forms occasionally appear which, particularly when tangled, resemble streptothricial organisms; they do not occur regularly enough to seem characteristic features of any particular medium.

INFLUENCE OF SUGAR AND MEDIUM ON AMOUNT OF GROWTH

The amount of growth in these sugar media varies widely, and the effect of the same sugar differs remarkably, depending on the type of medium. In the bouillons the growth is usually heaviest in those in which acid production is most marked and the lighter growths occur with those sugars not affected. Thus in dextrose bouillon, for instance, the growth is very heavy, with a coarse, flocculent deposit on the bottom and the sides. On dextrose agar, however, the reverse holds true, the growth here being very quickly inhibited by the acid produced. In the bouillons dextrose, mannite, and levulose have given the heaviest development; maltose, dextrin, and galactose somewhat less; while only fair amounts of growth occurred in the remaining sugars except amygdalin, sorbite, and glycerin, which seemed distinctly unfavorable. These differences were more marked after three than after ten days.

With the agar media, growth seems promptly to cease once the underlying medium becomes acidified. If this occurs early, the growth is very light; if later, it is correspondingly heavier. Whenever reversion to neutral occurs, the growth, having been temporarily retarded, goes on to maximum. Due to the operation of this rule the growths of the series I maltose set, in particular, varied widely.

In none of the serum waters has the growth appeared heavy, though from the nature of the material it is difficult to observe this feature with accuracy.

ANALYSIS OF FERMENTATION REACTIONS

Consideration of the inconstant and conflicting reactions obtained by the use of certain of these media brings up the question of the several factors concerned in the irregularities. These

are of more or less interest and importance, for while with some organisms fermentation is so active and definite that results are clear-cut under usual conditions, others, among which *B. pestis* is to be included, are less active in this respect and are, therefore, prone to irregularities of result unless the conditions of experiment are properly controlled.

Different types of media necessarily present different conditions, such as essential suitability, aëration, availability of contained reagent, and diffusion of end product, all of which may affect the metabolism of the organism under consideration. Beside these, differences in indicators must also be considered. In order to demonstrate the influences of certain of these various factors, a considerable number of tests have been carried out.

THE INFLUENCE OF TYPE MEDIUM ON FERMENTATION

The tests described above demonstrate that for *B. pestis*, which in most instances is a weak fermenter, the nature of the medium used plays an important rôle. Comparison of the numbers of sugars fermented in each type emphasizes the superiority of bouillon, as has repeatedly been shown for other organisms. Litmus-agar slants under certain conditions are almost as useful, but Hiss's serum water is of much less value, and the results with it are more misleading. These comparisons are summarized in Table IV, which is compiled from the results already shown together with certain reactions yet to be discussed.

TABLE IV.—*Summary of carbohydrate fermentation by B. pestis in various media, with 0.5 and 1 per cent concentration of sugars.*

[Symbols indicating degree of acid production: ++=strong and constant; +=definite and usual; ±=weak and irregular but usual; ∓=unusual and weak; —=none.]

Medium.	Litmus serum-waters.		Litmus agars.		Bouillon (titrations).		Remarks.
	0.5 per cent sugars.	1.0 per cent sugars.	0.5 per cent sugars.	1.0 per cent sugars.	0.5 per cent sugars.	1.0 per cent sugars.	
Dextrose	++	-------	++	-------	++	-------	Strong.
Mannite	++	-------	++	-------	++	-------	Do.
Levulose	±	+	+	-------	++	-------	Do.
Maltose	∓	+	±	+	++	++	Moderate.
Arabinose	(∓ ?)	+	—	+	+	+	Weak.
Galactose	—	∓	—	±	+	+	Do.
Salicin	—	±	—	+	+	+	Do.
Glycerin	—	—	∓	∓	∓	∓	Irregular.
Dextrin	—	—	—	-------	(± ?)	(± ?)	Negative.

Tests with the following were clearly negative: Lactose, adonite, inosite, saccharose, dulcite, sorbite, raffinose, amygdalin, nutrose, and inulin.

From this summary may be drawn definite conclusions as to the fermentation activities of *B. pestis* in the media used.

LIMITATION OF ACID TOLERANCE

In the 0.5 per cent bouillon series (Table III) the cultures, irrespective of the nature of the sugar, the extent of growth, or the primary acidity, showed a maximal acidity of about 2.5 per cent, which reaction was obtained with several sugars. This indicates a rather remarkably constant limit of acid tolerance for the various strains.

The influence of concentration of carbohydrate on end reaction.—That increase of sugar, up to a certain point at least and under certain conditions, causes an increase in the rapidity or the degree of the reaction is axiomatic. The effect of variation in amount depends, to a certain extent at least, upon the activity of the organism against the particular sugars used. For instance, 0.5 per cent dextrose serum-water was as completely acidified as was the 1 per cent medium. On the other hand, 1.5 or 2 per cent maltose or arabinose serum-waters would probably have reacted more strongly than did those of 1 per cent concentration to be shown in Table VI. In order to demonstrate whether widely different concentrations of sugar would have any influence on the final acidity, a series of peptone-water cultures was made, dextrose and mannite being the sugars used. Three strains of organisms with somewhat different fermentation activities were selected. The titration results appear in Table V.

TABLE V.—*Final acidity produced in peptone waters containing different amounts of carbohydrates.*

	Strain—			Control (average of two).
	B.	G.	I.	
Dextrose:				
0.5 per cent	2.00	1.72	1.92	0.68
1.0 per cent	2.16	1.80	1.88	0.76
1.5 per cent	2.16	1.92	2.04	0.84
2.0 per cent	2.24	1.84	1.92	0.88
2.5 per cent	2.16	2.00	2.12	1.00
Mannite:				
0.5 per cent	1.76	1.32	1.44	0.54
1.0 per cent	1.68	1.32	1.48	0.50
1.5 per cent	1.8	1.32	1.44	0.52
2.0 per cent	1.75	1.40	1.00	0.44
2.5 per cent	1.84	1.32	1.48	0.48

These results show that a considerable variation in concentration of sugars has no appreciable effect on the total acid produced and that here, as elsewhere, the acid production by any one strain with the same sugar is remarkably constant. There is again demonstrated, however, a consistent although slight difference among the strains.

In order to obtain direct evidence as to whether the acidities attained in the 0.5 series had in any degree been determined by the low sugar concentration, a number of 1 per cent sugars was similarly tested. To learn, further, whether a simpler nutrient vehicle for the carbohydrates would modify the amount of acid produced, a small series was also tested in a solution containting 2 per cent peptone and 0.5 per cent sodium chloride. The results of these series are included in Table VI.

TABLE VI.—*Total acidities attained in 1 per cent carbohydrate fluid media, in terms of cubic centimeters of normal sodium hydroxide.*

Strain.	Bouillon 1 per cent.[a]					Peptone waters, 1 per cent.[a]			
	Levulose.	Maltose.	Salicin.	Arabinose.	Galactose.	Maltose.	Salicin.[c]	Arabinose.	Galactose.
A	2.15	1.7	1.7	2.0	1.85	2.3	0.75 (0.05)	2.5	2.4
B	2.3	1.5	1.8	2.0	1.9	2.5	1.15 (0.45)	2.65	2.4
C	2.2	1.7	2.0	2.2	1.8	0.95	0.75 (0.05)	2.75	2.2
D	2.2	1.55	1.7	2.05	1.85	1.5	0.75 (0.05)	2.65	2.3
E	2.1	1.6	1.25	2.1	1.8	2.5	0.95 (0.25)	2.45	2.35
F	(b)	1.65	2.3	1.95	1.65	1.9	0.9 (0.20)	2.0	2.3
G	1.8	1.45	1.2	1.7	1.3	1.35	0.85 (0.15)	1.95	1.55
H	2.1	1.45	1.0	2.05	1.7	2.35	0.7 (0.0)	1.85	1.45
I	2.1	1.65	(b)	2.1	1.75	2.35	0.85 (0.15)	2.2	2.1
J	2.0	1.2	1.15	1.35	1.85	1.3	0.9 (0.20)	1.95	2.5
Controls:									
1	1.00	1.00	0.55	1.25	0.80	1.00	0.65	1.30	1.10
2	1.00	1.00	0.45	1.15	0.90	0.90	0.70	.....	1.15

[a] Titrated with test N/50 sodium hydroxide.
[b] No growth.
[c] Figures in parentheses represent apparent increase in acid.

Comparison of these results with those given in Table III shows that in no case was there a radical increase of acidity in the 1 per cent series. Contrasting the bouillon and peptone

cultures, it seems that fermentation in the peptone media was usually rather more active than in the corresponding bouillon. Of interest, however, is the fact that salicin fermentation in peptone water is remarkably depressed, this being similar to the results obtained in serum water. The uniformity in the amount of acid formed in the same medium by the different strains is again well shown. There is sometimes, however, a distinct difference in the end reaction in the different sugars, an indication, possibly, of different end products which are inhibitory in different concentrations.

Influence of primary acidity on end reaction.—In connection with inhibition of fermentation the question arises whether a simple primary acidity, due to a known substance, might not be tolerated to a higher degree than the mixed products of bacterial metabolism. To investigate this point, two series of cultures of different reactions were made by adding hydrochloric and lactic acids to neutral broth. Dextrose and mannite were again used for the carbohydrates. The results of these titrations indicated that fermentation is inhibited as quickly by hydrochloric or lactic acid as by the products of the organism's own metabolism. No difference whatever was demonstrated in the tolerance to the two acids.

Acid limit with meat-infusion broths.—In the foregoing titrations the maximal acidity attained was between 2.5 and 3 per cent to phenolphthalein. This is not the highest total acidity tolerable to *B. pestis*, for cultures in sugar broths made up of sugar-free veal infusion attain a considerably higher acidity than has been the case in beef-extract broths under any circumstance.

TABLE VII.—*Final reactions attained in 1 per cent veal-infusion sugar broths.*

Strain.	Dextrose.	Mannite.	Maltose.
A	4.0	4.2	3.5
B	4.1	4.0	4.2
C	4.2	4.2	2.2
D	4.2	4.1	4.0
E	4.2	3.8	3.7
F	3.8	3.8	4.0
G	4.2	4.1	3.5
H	3.8	4.1	3.8
I	4.0	3.5	3.7
J	4.0	4.3	2.1
Control titrations:			
1	1.6	1.3	1.7
2	1.3	1.3	1.7

The figures in Table VII demonstrate that *B. pestis* in the veal-infusion broths tolerates acid up to about 4 per cent (3.8 to 4.2), which is 1 to 1.5 per cent higher than in beef-extract media.

THE INFLUENCE OF TEMPERATURE AND OXYGEN PRESSURE ON RATE OF FERMENTATION

It is a recognized fact that the temperature at which *B. pestis* is cultivated influences the organism to a considerable degree. In consideration of this the rates of acid production at incubator and room temperatures were compared. In Table VIII are shown the end reactions in 1 per cent Hiss's serum waters containing levulose, maltose, salicin, arabinose, galactose, and glycerin. The room temperature ranged from 27 to 30° C. during the period of cultivation.

TABLE VIII.—*Comparison of fermentation of 1 per cent sugar serum-waters at incubator and room temperatures.*

[End result after ten days; symbols as in Table I; the day of observation in parentheses.]

Strain.	Levulose.		Maltose.		Salicin.		Arabinose.		Galactose.		Glycerin.	
	37°.	28°.	37°.	28°.	37°.	28°.	37°.	28°.	37°.	28°.	37°.	28°.
A	4+ (4)	4+ (3)	4+ (5)	4+ (3)	+ (10)	+ (4)	4+ (4)	4+ (9)	2+ (3)	4+ (4)	—	—
B	4+ (3)	4+ (3)	3+ (9)	4+ (4)	± (4)	+ (6)	2+ (5)	4+ (4)	+ (2)	4+ (10)	—	—
C	4+ (3)	4+ (3)	1+ (4)	4+ (3)	—	3+ (9)	2+ (9)	4+ (9)	+ (2)	2+ (9)	—	—
D	4+ (3)	4+ (3)	4+ (3)	4+ (2)	± (4)	3+ (9)	4+ (4)	4+ (3)	+ (2)	4+ (5)	—	—
E	3+ (7)	4+ (3)	1+ (4)	4+ (3)	—	+ (6)	+ (4)	4+ (5)	+ (3)	2+ (5)	—	—
F	4+ (4)	4+ (3)	1+ (4)	4+ (2)	1 (9)	4 (7)	+ (5)	4+ (5)	+ (2)	+ (3)	—	—
G	+ (3)	4+ (4)	1+ (8)	4+ (4)	—	—	2+ (9)	4+ (6)	+ (5)	2+ (9)	—	+ (8)
H	4+ (3)	4+ (3)	4+ (3)	4+ (3)	—	± (4)	4+ (4)	4+ (4)	+ (3)	+ (3)	+ (4)	+ (5)
I	2+ (5)	4+ (3)	1+ (4)	4+ (2)	± (10)	+ (8)	+ (5)	4+ (5)	+ (2)	+ (3)	—	—
J	+ (8)	4+ (3)	4+ (5)	4+ (3)	—	—	—	± (3)	+ (3)	4+ (4)	—	—

The serum-water cultures represented in Table VIII evidenced a considerably greater activity at the room temperature, at which saprophytism is pronounced, than at 37° C., the temperature of human-body parasitism.

Similar, though somewhat less marked, is the difference in effect of these temperatures with litmus agars. In practically every instance acidification began more quickly on the room-temperature slants and in most instances became complete sooner. Further, in several cases it became complete only at room temperature. With 0.5 per cent agars several maltose cultures in the incubator became partially acid and reverted to neutral, while the corresponding room-temperature cultures became and remained strongly acid.

Two sets of agar slants, maltose and glycerin, were cultivated in vacuo at body temperature in a Novy jar. The reactions were at first accelerated to equal those of the room-temperature control cultures, but at the end of a week the cultures showed little difference from the aërobic incubator controls. Later duplicate sets of 1 per cent maltose serum-water and litmus agar were similarly cultivated. Here there was definitely, although but slightly, more active fermentation in the anaërobic sets, the advantage persisting through the four days for which the cultures were observed.

FERMENTATION IN HISS'S LITMUS SERUM-WATER

Effect on medium of directly added acids.—Comparison of the reactions of *B. pestis* in serum-water cultures and on litmus agars is distinctly unfavorable to the former, though it would seem that, so far as the indicator is concerned, the results should coincide. The possibility that a so-called "buffer" absorption of acid by the serum itself might play some part in the delayed reaction was considered. To determine this, increasing amounts of dilute hydrochloric and lactic acids were slowly added to measured volumes of sugar serum-waters, the tubes being agitated the while. The results were practically identical in several tests. The amounts of acids required to produce the changes are small and regular, and the "buffer" effect is, at the most, but 0.1 to 0.2 per cent. The acidity required to produce various stages represented heretofore by the "plus system" is shown in Table IX. By the application of these equivalents, the readings in Tables I and VIII might be made roughly quantitative.

TABLE IX.—*Changes produced by adding dilute acids to Hiss's litmus serum-water, as determined with hydrochloric and lactic acids.*

Normal acid.	Appearance with transmitted light.	Appearance with reflected light.	Equivalent to symbols as used.
Per cent.			
0.1	None, or faintest perceptible change	No change	—
0.2	Slight reddening	No change	±
0.3	Red predominent	Faint reddening	±
0.4	Red almost pure	Reddening more distinct	+
0.5	(About as above) faintest clouding	(About as above)	+
0.6	Red practically pure; opacity developing.	Red and blue about equal; precipitation not detectable.	2+
0.7			2+
0.8	Red and opaque, but fluid	Red practically pure; opacity evident.	3+
0.9	Coagulated		3+
1.0	Coagulated	Coagulated	4+

Acid production in serum bouillon.—Another question to be determined was whether unsatisfactory results with serum waters were due to limitation of growth on account of the simplicity of the medium, or to the possibility that the serum itself actually exercises a restraining effect. If the latter were the case, sugar peptone-water with varying concentrations of added serum should show differences in fermentation. A quantity of dextrose peptone-water was separated into five lots, to four of which Berkefeld-sterilized horse serum was added in various concentrations. These and a control set without serum were inoculated, using all ten strains of *B. pestis*. Titrations were made with N/40 sodium hydroxide, after six days' incubation in this case. The results appear in Table X.

TABLE X.—*Acid production in dextrose peptone-water containing various amounts of filter-sterilized horse serum.*

Strain.	Serum.				Control without serum.
	0.2%.	1.0%.	5.0%.	10.0%.	
A	2.65	2.5	2.75	2.5	2.6
B	2.6	2.6	2.55	2.6	2.3
C	2.6	2.55	2.75	2.8	2.65
D	2.5	2.55	2.65	2.65	2.55
E	2.2	2.4	2.65	2.35	2.25
F	2.4	2.65	2.7	2.5	2.25
G	2.5	2.75	2.6	2.4	2.5
H	2.65	2.75	2.75	2.8	2.25
I	2.2	2.2	2.5	1.8	2.0
J	2.35	2.4	2.65	2.5	2.15
Uninoculated controls	0.6	0.6	1.05	1.3	0.65
	0.75	0.8	----------	0.95	0.19

No depression of fermentation is demonstrated in these cultures, indicating that serum per se, in these percentages at least, is not inhibitory, despite the fact that the amount of growth was somewhat less than in dextrose peptone-water without serum.

Influence of added nutrient on the reaction with Hiss's serum waters.—Since serum per se appears not to have an inhibitory effect, the effect of adding simple nutrient substances to the serum-water stock was investigated. Beef extract alone proved to be of no value. Peptone, if added in 1 per cent concentration to the previously heated serum, gives, upon reheating, a soft jelly, as described by Buerger,(19) which physically is not the same as Hiss's medium. Making the original serum water one part of serum to four of water and adding 0.5 per cent of peptone after the primary heating gives an enriched medium which is

similar to Hiss's in appearance and reaction. Its value, however, seems but moderately greater than the ordinary serum water. With some sugars, as levulose and maltose, the reaction in the peptonized cultures was considerably accelerated, but with galactose the two media gave identical results, and with salicin no fermentation occurred in either.

SUMMARY AND DISCUSSION

Cultivations carried out with ten strains of *B. pestis* in media containing twenty fermentable substances have demonstrated conclusively the fermentation activities of the organism. Experiments have also demonstrated the parts played by certain influencing factors in the results obtained. As shown in Table IV, dextrose, mannite, and levulose are regularly and strongly fermented, while maltose, arabinose, galactose, and salicin are less constantly acted upon except in the more suitable media. Glycerin, which Vourland and Schöbl found to be fermented, in my experience reacted with but three strains out of ten, and then only under suitable conditions. This difference between the strains seems to have no significance. Contrary to the conclusion of MacConkey, dextrin is not fermented; contrary, similarly, to Schöbl and to Vourland, saccharose remains untouched. Salicin fermentation is peculiar in that it is practically negligible in serum water and in simple peptone media, although positive in agar and bouillons.

The strains used included a culture isolated in 1912 from a rat in New Orleans, three from human cases in the 1914 New Orleans invasion (suggested at the time by the Federal authorities to have come from Liverpool), five isolated from cases in the Philippine Islands, and one avirulent strain. Between these there has been demonstrated no distinct difference except for the irregularity with glycerin noted and except that between the strains there are consistent although slight differences in acid tolerance. This is shown numerically in Table III, where the relative "activities" of the strains in bouillons have been averaged. Strains *G* and *H* produce somewhat less, and strains *A* and *I* slightly more, acid than the majority. These variations are evidently not dependent upon length of time out of the animal body. The relation to virulence has not been investigated, but it is probably true that, as recently asserted by Berlin, this feature, also, has no definite influence.

There has been demonstrated a fairly constant point of maximum acid tolerance beyond which active metabolism as expressed in fermentation ceases. This is a well-recognized feature

of bacterial fermentation. *Bacillus coli*, for instance, as shown by Browne,(20) has a similarly uniform maximal point, which lies between 2.1 and 2.4 per cent in dextrose and somewhat lower with some sugars and is not raised by increase in sugar present, or in the total amount of medium, or by increase in initial acidity. Streptococci are quite different in that the maximal point of acid toleration differs greatly in different strains. Broadhurst's figures,(21) obtained from sugar-free, meat-infusion cultures, ranged between 1.5 and 8.5 per cent. In a beef-extract broth series(22) the highest that she obtained was 2.3 per cent as compared with 5.0 and 5.2 per cent with parallel infusion broths, though with meat-extract broths Fuller and Armstrong(23) obtained much higher figures. Hopkins and Lang(24) concluded that—

> Fermentation by a given streptococcus ceases when a certain acidity is reached, irrespective of how much acid must be formed to produce this acidity.

So far as can be learned, the maximum acid production by *B. pestis* has not previously been noted, although the limit of primary acidity of media permitting the growth of the organism has been studied. Wladimiroff and Kressling(25) found that the addition of small amounts of acid diminished the amount of growth, 3 per cent of normal hydrochloric acid causing complete inhibition. Lactic acid was better tolerated, 5 per cent being the maximum. Pakes and Joseph(26) found the point of inhibition of growth to be as high as 4.0 to 4.5 per cent. My results, using sugar bouillons of different degrees of acidity, do not coincide with this, as there was little growth and no increase in end reaction with media primarily 2.5 per cent acid or above, hydrochloric and lactic acids having identical limits.

It is shown that the maximum acidity produced by *B. pestis* in beef-extract bouillons ranges between 2.5 and 3 per cent. This seems to be the same for all strains, although (see particularly Table V) a strain may have a slightly lower maximum point with one sugar than with another, due possibly to the special nature of the end products. In some cases (see Table VI) all strains uniformly show this feature, which has often been noted in carbohydrate-fermentation studies.

Similarly to Broadhurst's results with streptococci, veal-infusion broths permit, for some reason not determined, a greater accumulation of titrable acid before fermentation is inhibited. This seems probably due to differences in the nature of secondary end products. The figures obtained in the series detailed in

Table VII show the maximal point under these conditions to be between 3.5 and 4.1.

Beyond the point of sugar concentration required under the conditions obtaining to bring the total acid produced up to the point of tolerance, addition of more sugar does not increase the final acidity. In several instances (see Table IV) 0.5 per cent of reagent has proved insufficient to secure maximum fermentation. Further, in a number of instances (as in Table II) reversion to neutral from incomplete acidification of 0.5 per cent agar slants occurred.

Comparative cultivations show that room temperature, which was used by Schöbl in his tests, influences fermentation to considerably greater activity than does body temperature. Anaërobic cultivation at the latter heat accelerates, at least temporarily, acid formation sometimes even more than does aërobic cultivation at room temperature.

The morphology of the organism varies more or less with the sugars, depending not only upon the involuting and degenerating influence of the acid sometimes formed, but also upon other less evident influences where acid is not produced. The extent of growth is also considerably modified, apparently anomalously in different media. In bouillons containing fermentable substances the growth is most luxuriant; on agars containing the same reagents the growths are much less heavy than on unacidified media. In bouillon, because of its fluidity, acid when formed cannot inhibit growth until such time as the entire mass of the medium is brought to the maximum point of acid tolerance. In agar, on the other hand, as soon as the medium directly beneath the growth becomes sufficiently acidified, multiplication seems almost to cease. Further production of acid constantly replaces that lost by diffusion to the deeper levels, and the growth remains light. Thus under different physical conditions the same carbohydrate produces opposite effects. Occasionally, when acid production in the agar culture is weak, reversion occurs and the primary, light, acid-producing growth is replaced by a heavy, nonfermenting growth.

Of the three methods of determining acid production, titration of sugar bouillons is by far the best, as is shown particularly by the contrast between the negative or weak and irregular fermentation of salicin, arabinose, galactose, and maltose in the other media and their regular fermentation in bouillons. Only in bouillons did the 0.5 per cent series approximate the 1 per cent series in regularity or intensity of reaction. In sugar

peptone-waters a slightly greater amount of acid sometimes developed than in the corresponding bouillons.

The degrees of acidity required to show change in litmus serum-waters may be demonstrated by adding very dilute normal acids to measured quantities of the medium. Acid up to or slightly above the equivalent of 0.1 per cent usually causes no reaction, an inertia due possibly to the "buffer" effect of serum, which Levy and Rowntree(27) found to be from 0.1 to sometimes 0.3 per cent for fresh serum, which amount of acid could be added without raising the hydrogen ion concentration. This margin is too slight to be responsible for the occasional apparent fermentation inactivities in serum media. Faint change usually occurs with between 0.1 and 0.2 per cent acidification, while coagulation is complete with less than 1 per cent, the reactions being identical with hydrochloric and lactic acids. The different changes are brought about regularly in different lots of the medium by fairly definite amounts of acid, which makes the observation of such reactions of approximate quantitative value up to the point of coagulation of the serum.

The unmodified litmus serum-waters of Hiss, although valuable in the identification of intestinal and of other organisms, have repeatedly proved unreliable and misleading with *B. pestis*. Similar experiences have been had by other observers with bacteria of low fermentation activity, as for instance certain streptococci. That the reactions obtained do not result from a directly inhibitory effect of the serum is indicated by the fact that no suggestion of inhibition was shown in bouillons to which serum had been added. Further, the addition of peptone sometimes increases the reaction, although this acceleration is not constant enough with the different sugars to make such a medium of general value in a study of bacterial fermentation.

CONCLUSIONS

Comparison has failed to demonstrate any distinct difference, qualitative or quantitative, between the fermentation activities of Oriental (Philippine) and certain American strains of *B. pestis*. There is, on the other hand, a rather remarkable agreement between the different strains except solely with regard to glycerin fermentation.

Under usual conditions dextrose, mannite, and levulose are fermented regularly and fairly strongly. Maltose, arabinose, galactose, and salicin are also fermented, but more irregularly except under favorable conditions. A few strains ferment glycerin. Dextrin, lactose, saccharose, raffinose, adonite,

dulcite, amygdalin, inosite, sorbite, nutrose, and inulin are not changed. The division of the group into glycerin fermenters and nonglycerin fermenters has no apparent significance.

Veal-infusion bouillon is the most suitable medium for carbohydrate fermentation by *B. pestis*. Litmus agar is more favorable than Hiss's litmus serum-water unmodified, which is quite unsuitable. Diminished reaction in Hiss's serum-waters is due to unsuitability of the medium for luxuriant growth of the organism and not to any directly inhibiting effect of serum per se. In it, whether plain or peptonized, such reagents as appear to be not adapted to promote growth by being themselves primarily utilized are fermented secondarily and less decisively. Salicin seem peculiarly unfermentable by *B. pestis* in serum water or peptone water in usual concentrations.

The types of media and the different sugars used have various effects, some of them very definite and constant, on the amount of growth and on the morphology of the organism.

Fermentation ordinarily occurs more rapidly and completely at room temperature (27° to 30° C.) than at body temperature. Under anaërobic conditions at 37° C. acid production is accelerated, temporarily at least, to exceed the aërobic, room-temperature reactions.

There is a well-defined maximal point of acid tolerance, which is fairly uniform in the same medium for the different strains. This ranges from 2.5 to 3 per cent acid for beef-extract broths and 3.8 to 4.2 per cent with veal-infusion broths. There are slight differences in the highest acidities with the different carbohydrates. The maximal point is not changed by increased percentage of the sugar or differences in the original reaction.

REFERENCES

(1) Gioso and Biginelli. *Riv. d'igiene* (1898) (quoted by Dieudonné and Otto, reference below).
(2) Rees, D. C. *Brit. Med. Journ.* (1900), 2, 1236.
(3) Dieudonné, A., und Otto, R. Kolle u. Wassermann's Handbuch der pathogenen Mikroorganismen, Jena, 2d ed. (1912), 4, 173.
(4) Calvert, W. J. *Circ. Trop. Dis.*, Manila (1901), No. 3.
(5) Herzog, M. Plague, etc., *Pub. Bur. Govt. Lab.*, Manila (1904), No. 23.
(6) MacConkey, A. T. *Journ. Hyg.* (1905), 5, 333.
(7) Wherry, W. B. *Journ. Inf. Dis.* (1905), 2, 577.
(8) Idem. Ibid. (1908), 5, 485.
(9) Vourland. *Centralbl. f. Bakt. etc., Orig.* (1908), 45, 193.
(10) MacConkey, A. T. *Journ. Hyg.* (1908), 8, 335.
(11) Reports on plague investigation in India, issued by the Advisory Committee. *Journ. Hyg.* (1908), 8, 302.

(12) McCoy. G. W. *Journ. Inf. Dis.* (1909), 6, 170.
(13) Rowland, S. *Journ. Hyg.* (1910), 10, 536.
(14) Zabolotny, D. Rept. Internat. Plague Conf., Manila (1912), 42 and 68.
(15) Strong, R. P. Ibid. (1912), 433.
(16) Strong, R. P., and Teague, O. *Phil. Journ. Sci., Sec. B* (1912), 7, 187.
(17) Berlin, H. *Hamburg. med. Übersicht* (1914), 210. Abst. in *Centralbl. f. Bakt. etc., Ref* (1915), 63, 70.
(18) Wade, H. W., and Staring, H. L. *New Orleans Med. & Surg. Journ.* (1914–15), 67, 95.
(19) Buerger, L. *Journ. Exp. Med.* (1905), 7, 497.
(20) Browne, W. W. *Journ. Inf. Dis.* (1914), 15, 580.
(21) Broadhurst, J. Ibid. (1912), 10, 272.
(22) Idem. Ibid. (1913), 13, 402.
(23) Fuller, C. A., and Armstrong, V. A. Ibid. (1913), 13, 442.
(24) Hopkins, J. G., and Lang, A. Ibid. (1914), 15, 61.
(25) Wladimiroff and Kressling. (1897) Quoted by Albrecht and Ghon, Über die Beulenpest in Bombay im Jahre 1897, Wien (1898), II C, 608.
(26) Pakes, W. C. C., and Joseph, F. H. *Brit. Med. Journ.* (1905), 1, 136.
(27) Levy, R. L., and Rowntree, L. G. *Arch. Int. Med.* (1916), 17, 523.

REVIEWS

The Mortality | from Cancer throughout | the World | by | Frederick L. Hoffman, LL. D., | F. S. S., F. A. S. A. | [5 lines] | Newark, New Jersey | The Prudential Press | 1915 | Cloth, pp. i–xv + 1–826.

The following is an excerpt from the preface by the author:

"The work is divided into nine chapters, to all but one of which there is an appendix of forms or tables, which, as a matter of convenience, have been placed together at the end of the volume. Chapter I, on The Statistical Method in Medicine, is amplified by an appendix of the principal cancer classifications, past and present, used in standard textbooks and in the compilation of international cancer mortality statistics. * * * Chapter II, on The Statistical Basis of Cancer Research, is a brief discussion of the fundamental statistical facts available for analysis, enlarged by an appendix of the blanks and certificates used in connection with cancer mortality investigations and special research, including the question form for cancer census purposes recommended by the International Association and the special blanks for supplementary inquiries into the facts and circumstances connected with the occurrence of cancer of the uterus, mammary cancer, gastric cancer and cancer of the buccal cavity, adopted and recommended by the Statistical Committee of the American Society for the Control of Cancer, in coöperation with the General Memorial Hospital of the City of New York. Chapter III, on The Increase in Cancer, is an extended discussion of the general problem of the observed upward tendency of the cancer death rate throughout the world. * * * The Mortality from Cancer in Different Occupations is discussed in Chapter IV, with an appendix of eight tables of the mortality from cancer in selected industries and employments, derived from the decennial reports of the Registrar-General of England and Wales, but rearranged and recalculated for the present purpose. In addition, the appendix includes cancer mortality data by occupations, derived from the industrial mortality experience of The Prudential and the cancer census of Hungary. Chapter V presents an extended discussion of Cancer as a Problem in Life Insurance Medicine, historically and practically considered, with an appendix of 121 tables, including a concise and uniform presentation of the general cancer experience data of a large number of American and foreign life insurance companies and the collective results of the Medico-Actuarial Mortality Investigation.

Chapter VI, on the Geographical Incidence of Cancer Throughout the World, brings out forcibly the wide range in the cancer frequency rates of different countries and cities with widely varying circumstances of race, climate, habits, etc., all of which are shown to have an important bearing upon the cancer problem as a whole. * * * In Chapter VII, on The Statistical Data of Cancer Frequency in American States and Cities, the rate of cancer occurrence throughout the United States is discussed at some length, and amplified by an appendix of 259 tables of cancer mortality for the registration area and for the several states and cities in a uniform manner and with a due regard, as far as practicable, to the elements of age, sex, race, organs and parts, etc. Chapter VIII presents the corresponding information on The Statistical Data of Cancer Frequency in Foreign Countries, with an appendix of 389 tables for countries other than the United States. Chapter IX concludes the results of the statistical inquiry with Some General Observations and Conclusions on the Cancer Problem. This is a general discussion of practically all the more or less controversial aspects of the cancer question, with a first regard, however, to sociological, anthropological and general scientific consideration. * * * The appendix to this chapter includes reprints of suggestive educational circulars used in connection with the nation-wide propaganda for cancer control under the auspices of the American Society for the Control of Cancer, etc."

A bibliography of the important works and articles on cancer is included.

Differential diagnosis| volume II | [2 lines] | by | Richard C. Cabot, M. D. [3 lines | ornament | 1 line] | profusely illustrated | Philadelphia and London | W. B. Saunders Company | 1915 | Cloth, pp. 1–709. Price, $5.50; half morocco, $7.00.

Cabot's Differential Diagnosis, Vol. ii, has filled a long-felt want in differential diagnosis. Exhaustive analyses on the frequency of certain symptons, with diagnosis, have been made by the author. Innumerable cases are cited to illustrate these symptoms or symptom complex. This volume will be found to be of great help both to the clinician and to the student.

P. GUTIERREZ.

Painless Childbirth | Eutocia and | Nitrous Oxid-oxygen Analgesia | by | Dr. Carl Henry Davis | [4 lines] | [seal] | Chicago | Forbes & Company | 1916 | Cloth, pp. 1–134. Price, $1.

The first section of the book considers painless childbirth, discussing the departure under conditions of civilization from the ease of delivery said to exist in savagery, and justifies

the use of anesthetics. After sketching the development of anesthetics, it compares, very favorably to the latter, the pharmacology and the results of the practical use of the morphin-scopolomin mixture (known as "twilight sleep") and of nitrous oxide and oxygen in obstetrical work.

The second section, entitled Eutocia, considers certain unsatisfactory features of the general conditions that surround the present-day practice of obstetrics in the home and the hospital. A strong case is prepared statistically to show that the maternal death rates are, even with modern knowledge of asepsis, anesthetics, and obstetrical technique, unnecessarily high. This mortality seems largely due to puerperal infections which, under proper conditions, are avoidable.

The third part deals with nitrous oxid-oxygen analgesia in obstetrics. The results of practice in the Presbyterian Hospital of Chicago are analyzed to show the advantages of the analgesia over ordinary labor without anesthetics. The technique of the analgesia is considered, its simplicity, which even permits the patient to administer her own gas, is emphasized, and certain pertinent points for caution are noted.

This unpretentious little monograph seems clearly to point the way to a safe and practicable means of relieving the parturient woman of much of the suffering and nerve shock that ordinarily accompanies the condition. This is in line with the extension of the use of this analgesia in dental and in certain simpler surgical operations.

H. W. W.

A Handbook | of | Infant Feeding | by | Lawrence T. Royster, M. D. | [5 lines] | illustrated | St. Louis | C. V. Mosby Company | 1916 | Cloth, pp. 1–144. Price, $1.25.

Within recent years a good number of handbooks have been written on infant feeding. Many of them have justified their publication, while others seem to be somewhat superfluous. In the little volume under review the author has attempted to furnish the busy practitioner, in "a compact and succinct form," with the essential and practical side of infant feeding, leaving aside all the conflicting and theoretical points of this important subject of pediatrics.

The book contains fifteen chapters and one appendix, which includes the commentaries upon the various constituents of the food; the growth and development and the stools of infants; natural and artificial feeding; the care of premature infants; the digestive disturbance of both breast- and bottle-fed infants;

the feeding of difficult cases; the feeding during the second year; marasmus; infectious diarrhœa; the preparation of formulæ by the percentage method; the composition and preparation of the foods most employed, such as barley water, whey, casein milk of Finkelstein, buttermilk, and batter bread. There is also presented the Harvard classification of gastrointestinal disturbances. Worthy of mention as interesting features of the handbook are the following chapters:

1. The stools of infancy, masterly written by Professor Lorett Morse.

2. The bottle feeding gives valuable hints to modify cows' milk in order to reach "a gradual adaptation of the ingredients to the digestive power of infants."

3. The chapter on the digestive disturbances, in which the author wisely adopts the German division of disturbances in breast-fed and in bottle-fed infants.

4. The chapter on the handling of difficult cases of feeding also must be commended, as presenting very practical advice for the practitioner.

It is to be regretted that the chapter on the treatment of marasmus is so short and rather incomplete.

Altogether the book is a successful attempt to guide the practitioner, especially the American physician, who is very well acquainted with the principles of the percentage method, and to solve the daily problem of conducting the feeding of infants.

JOSÉ ALBERT.

Diagnostic Methods | [6 lines] | by | Herbert Thomas Brooks, A. B., M. D., | [2 lines] | third edition | revised and rewritten | St. Louis | C. V. Mosby Company | 1916 | Cloth, pp. 1–96. Price, $1.

This little book of diagnostic methods is very well adapted to the needs of the medical student, interne, and practicing physician. By referring to it, often many important and essential diagnostic facts may be kept in mind, thereby obtaining more vital data from each patient. Using this handbook as a guide and a large textbook for reference, a decided improvement in the character of medical work performed by the average practitioner should result.

The average textbook on diagnostic methods is altogether too exhaustive for everyday work, and the practicing physician finding it so formidable decides to depend wholly on his native knowledge of this subject. Undoubtedly this handbook will bridge over the gulf and will encourage the physician toward a more complete study of the individual case.

T. F. KEATING.

Candy | Medication | by | Bernard Fantus, M. D. | Professor of Pharmacology and Therapeutics College of | Medicine, University of Illinois, Chicago. | St. Louis | [ornament] | C. V. Mosby Company | 1915 | Cloth, pp. 1–82. Price, $1.

Candy Medication by Doctor Fantus presents a method for making the administration of medicine less obnoxious to children by using it in the form of candy. He gives formulæ for the use of fifty such medicaments. These fifty pretty well cover the gamut of children's ordinary ailments, and their general administration in the form of candy should be hailed as a distinct advance in robbing childhood of its dread of obnoxious medicine.

The book is well written and free from any serious objectionable features. It is a distinct addition to the literature of medicine dispensing and deserves the favor of the physician and pharmacist. The dose in most cases is small, being usually about one tenth the size of the average dose recommended by the United States Pharmacopœia. However, as the author states, the smallness of the dose is an advantage, since it necessitates frequent administration which is a good principle in practice, owing to the greater activity of the vital processes of the child.

H. C. BRILL.

Post-Mortem | Examinations | by | William S. Wadsworth, M. D. | Coroner's physician of Philadelphia | with 304 original | illustrations | Philadelphia and London | W. B. Saunders Company | 1916 | Cloth, pp. 1–598. Price, $6 net; half-morocco, $7.50 net.

Wadsworth's book contains much that is praiseworthy and not a little that may be criticized. The scope of a post-mortem examination is so great that it is a matter of some difficulty to decide what shall be included in, and what excluded from, a book devoted to the subject. The author is correct in his idea that a post-mortem operator should have a broad knowledge of the medical sciences, but it is manifestly impossible to inclose an encyclopædia of these sciences between the covers of one book. That part of the author's work that is devoted to description of technique is for the most part excellent, many original observations have been recorded, and the illustrations are well selected and beautifully executed. The intense personal element which everywhere pervades the text does not materially add to the value of a book which from its very nature should derive its greatest circulation among those without a large mortuary experience. A solved problem appears to have presented fewer difficulties than before its solution was reached, and post-mortem revelations are too common an incident to call for diatribes against every one but the post-mortem operator. Charity toward

men in the clinical branches would be more frequent if the "post-mortem operator" could see more of the doctor's cases that do not come into his hands. At any rate, the book is one which cannot be unconditionally recommended for medical students.

The publishers deserve very great credit for the appearance of the book.

B. C. C.

Pellagra | an American problem | by | George M. Niles, M. D. | [4 lines] | second edition | illustrated | Philadelphia and London | W. B. Saunders Company | 1916 | Cloth, pp. 1–261. Price, $3 net.

This book on pellagra is the second edition of one of the best American treatises on a condition which, previous to 1907 and 1908, was seldom recognized in the United States. Since that time it has increased and spread so alarmingly that it has become one of the serious American problems. Under these conditions, when much of the medical profession is unsatisfactorily informed as to the disease, a book such as that under review is particularly of value. It is intended for the physician and is written by a physician who has evidently been embarrassed in his efforts to keep in touch with all features of so many-sided a question as pellagra.

The work takes up the historic and other considerations; various phases of the etiology controversy are detailed; the symptomatology and clinical course are discussed and followed by an illustrative chapter of case reports; the pathology and morbid anatomy are briefly considered; the diagnosis, treatment, and prophylaxis are thoroughly discussed; and certain reports of animal experiments are added as a final chapter.

The book as a whole is written in a somewhat chatty style which often makes for pleasant reading, though occasionally it is carried so far as to detract from the seriousness of the work. The sections of the book which deal with phases familiar to the author exhibit a confidence and authority in contrast to other sections covering less familiar territory. The discussion of such subjects as diagnosis and treatment are valuable contributions in which the work of others is considered judicially, from the standpoint of much experience. Upon other features, however, the author sometimes contents himself with more or less extensive quotations and excerpts, outlining various hypotheses which are often antagonistic with little or no expression of opinion or personal experience.

The various theories of etiology are outlined, and a preponderance of evidence is shown to be in favor of the idea that pellagra

is a disease of dietary deficiency, in which the author believes that altered corn plays a leading rôle. In the discussion of the nutritive value of corn, it would seem that recent work on the chemistry of corn as a food has not been noted. Further, presumably because it was sent to press too early, Goldberger's last report, based on his Mississippi experiments, was not included. This is probably well, for the early reports of this work are inconclusive, and the author already lays sufficient emphasis on the dietetic hypotheses.

A separate, "somewhat supplementary chapter," which deals with "some recent experiments on animals, and deductions therefrom" is confined to two papers, one by Lavinder and the other by Anderson and Goldberger. It is now deficient as a summary of the subject, as it is evident that no attempt has been made to bring it to date in the new edition. Thus the latter article is still spoken of as "a recent bulletin," and the subsequent and apparently successful inoculation experiments of Harris, of New Orleans (1913), which at least are worthy of consideration, are ignored.

Occasionally there appear instances of careless editing, as where, in a quotation from a "recent" article (which was published in 1910), the word "root" is missing from "the posterior of the spinal nerves" (p. 159), or the word "flourescent" for "fluorescent" (p. 239) in a discussion of food substances. More noticeable is the evidence of hasty revision. Phrases such as "a recent case," a case seen "several months ago," or an advertisement which "the daily papers have recently carried" are inappropriate in a work intended to run for more than one edition. To speak of a personal report from a man "who has recently held four postmortems" (p. 163) and then add (p. 165) that "Since the first edition of this book was published * * * has performed," is, to say the least, unusual.

Such faults of style and revision as those suggested cannot but prejudice the reception of any work, and it is particularly unfortunate in this case as there is, in the body of the book, much material of value to the physician whose problem is the recognition and treatment of the disease.

H. W. W.